



SCHEMATIC REPRESENTATION OF THE PHAGEMID VECTOR pCES1

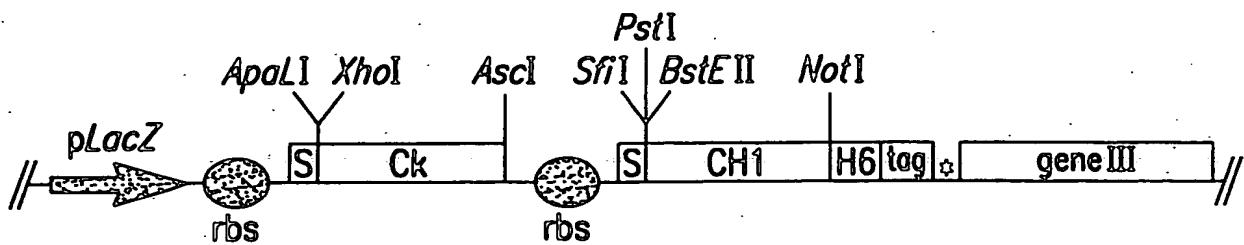


FIG. 1A



POLYLINKER REGION OF THE PHAGEMID VECTOR pCES1 (SEQ ID NO: 6)

Signal sequence *Xba*I +1
... TTA TTC GCA ATT CCT TTA GTT CCT TTC TAT TCT CAC AGT GCA CAG GTC CAA CTG CAG GTC GAC CTC GAG
L F A I P L V V P F Y S H S A Q V Q L Q V D L E

ApalI +1

Signal sequence

... TTA TTTC GCA ATT CCT TTA GTT CCT TAT TCT CAC AGT GCA CAG GTC CAA CTG CAG GTC GAC CTC GAG
L F A I P L V V P F Y S H S A Q V Q L Q V D L E

.ASCII

rbs

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The diagram shows the sequence of the Human CK gene. The sequence is: ATC AAA CGT GGA ACT GTG ... GGA GAG TGT TAA TAA GGC GCG CCA ATT CTA TTT CAA GGA GAC AGT CAT A. The gene is labeled "Human CK gene". A bracket labeled "Signal sequence" covers the sequence from the start (ATC) to the first restriction site (SfiI). A bracket labeled "rbs" covers the sequence from the first restriction site (SfiI) to the stop site (TAA). A bracket labeled "StopS" covers the sequence from the stop site (TAA) to the end (CAT A). An arrow labeled "(SEQ ID NO: 7)" points to the signal sequence. A bracket labeled "Human CK gene" covers the entire sequence from ATC to CAT A.

Signal sequence

Sfi I

ATG AAA TAC CTA TTG CCT ACG GCA GGC GCT GGA TTG TTA CTC GCG CCG GCC ATG GCC CAG GTG
 M K Y L L P T A A G L L A A Q P A M A Q V

BstEII PstI

Not I

PstI	BstEII	NotI
CAG <u>CTG</u> CAG GAG AGC <u>GGG</u> <u>GTC</u> ACC GTC TCA AGC GCC TCC ACC ...	TGT GCG GCC GCA CAT CAT CAT	
Q L Q E S G V T V S S A T	K S C A A A H H H H	

CAT CAC GGG GCA GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT GGG GCC GCA TAG ACT GTT ...
H H G A A E O K L I S E E D L N G A A * T V

GAT CTG AAT GGG GCC GCA
D L N G A A

Amber GeneIII

C-Myc Tag

(SEQ ID NO: 8)

1B
EIG

Oligonucleotide primers used for construction of the library

A. Primary amplifications

IgM heavy chain constant region

HuIgMFOR 5'-TGG AAG AGG CAC GTC CTT TTC TTT-3' (SEQ ID No:9)

κ light chain constant region

HuCKFOR 5'-ACA CTC TCC CCT GTT GAA GCT CTT-3' (SEQ ID No:10)

λ light chain constant region

HuCl2-FOR 5'-TGA ACA TTC TGT AGG GGC CAC TG-3' (SEQ ID No:11)

HuCl7-FOR 5'-AGA GCA TTC TGC AGG GGC CAC TG-3' (SEQ ID No:12)

B. Secondary amplifications

κ light chain constant region

HuCKFOR 5'-ACA CTC TCC CCT GTT GAA GCT CTT-3' (SEQ ID No:10)

λ light chain constant region

HuCl2-FOR 5'-TGA ACA TTC TGT AGG GGC CAC TG-3' (SEQ ID No:11)

HuCl7-FOR 5'-AGA GCA TTC TGC AGG GGC CAC TG-3' (SEQ ID No:12)

VH back

HuVH1B/7A-BACK	5'-CAG RTG CAG CTG GTG CAR TCT GG-3' (SEQ ID No:13)	HuVH1B/7A-BACK-SFI
HuVH1C-BACK	5'-SAG GTC CAG CTG GTR CAG TCT GG-3' (SEQ ID No:14)	HuVH1C-BACK-SFI
HuVH2B-BACK	5'-CAG RTC ACC TTG AAG GAG TCT GG-3' (SEQ ID No:15)	HuVH2B-BACK-SFI
HuVH3B-BACK	5'-SAG GTG CAG CTG GTG GAG TCT GG-3' (SEQ ID No:16)	HuVH3B-BACK-SFI
HuVH3C-BACK	5'-GAG GTG CAG CTG GTG GAG WCY GG-3' (SEQ ID No:17)	HuVH3C-BACK-SFI
HuVH4B-BACK	5'-CAG GTG CAG CTA CAG CAG TGG GG-3' (SEQ ID No:18)	HuVH4B-BACK-SFI
HuVH4C-BACK	5'-CAG STG CAG CTG CAG GAG TCS GG-3' (SEQ ID No:19)	HuVH4C-BACK-SFI
HuVH5B-BACK	5'-GAR GTG CAG CTG GTG CAG TCT GG-3' (SEQ ID No:20)	HuVH5B-BACK-SFI
HuVH6A-BACK	5'-CAG GTA CAG CTG CAG CAG TCA GG-3' (SEQ ID No:21)	HuVH6A-BACK-SFI

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FIG. 2i



Oligonucleotide primers used for construction of the library

VH forward

HuJH1/2-FOR
HuJH3-FOR
HuJH4/5-FOR
HuJH6-FOR

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Vκ back

HuVκ1B-BACK 5'-GAC ATC CAG WTG ACC CAG TCT CC-3' (SEQ ID NO:22)
HuVκ2-BACK 5'-GAT GTG ATG ACT CAG TCT CC-3' (SEQ ID NO:23)
HuVκ3B-BACK 5'-GAA ATT GTG WTG ACR CAG TCT CC-3' (SEQ ID NO:24)
HuVκ4B-BACK 5'-GAT ATT GTG ATG ACC CAC ACT CC-3' (SEQ ID NO:25)
HuVκ5-BACK 5'-GAA ACG ACA CTC ACG CAG TCT CC-3' (SEQ ID NO:26)
HuVκ6-BACK 5'-GAA ATT GTG CTG ACT CAG TCT CC-3' (SEQ ID NO:27)

FIG.2ii



Oligonucleotide primers used for construction of the library

V λ back	V λ back
HuV λ 1A-BACK	5'-CAG TCT GTG CTG ACT CAG CCA CC-3' (SEQ ID NO:28)
HuV λ 1B-BACK	5'-CAG TCT GTG YTG ACG CAG CCG CC-3' (SEQ ID NO:29)
HuV λ 1C-BACK	5'-CAG TCT GTC GTG ACG CAG CCG CC-3' (SEQ ID NO:30)
HuV λ 2-BACK	5'-CAR TCT GCC CTG ACT CAG CCT-3' (SEQ ID NO:31)
HuV λ 3A-BACK	5'-TCC TAT GWG CTG ACT CAG CCA CC-3' (SEQ ID NO:32)
HuV λ 3B-BACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3' (SEQ ID NO:33)
HuV λ 4-BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3' (SEQ ID NO:34)
HuV λ 5-BACK	5'-CAG GCT GTG CTG ACT CAG CCG TC-3' (SEQ ID NO:35)
HuV λ 6-BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3' (SEQ ID NO:36)
HuV λ 7/8-BACK	5'-CAG RCT GTG GTG ACY CAG GAG CC-3' (SEQ ID NO:37)
HuV λ 9-BACK	5'-CWG CCT GTG CTG ACT CAG CCM CC-3' (SEQ ID NO:38)

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FIG.2iii

Oligonucleotide primers used for construction of the library

5'-ACC GCC TCC ACC GGG CGG CGC GCC GCG CCG TTA TTA ACA CTC CCT GTT GAA GCT CTT-3' (SEQ ID NO:39)

5'-ACC GCC TCC ACC GGG CGG CGC GCC GCG CCG TTA TTA ACA TTC TGT AGG GGC CAC TG-3' (SEQ ID NO:40)

5'-ACC GCC TCC ACC GGG CGG CGC GCC GCG CCG TTA TTA AGA GCA TTC TGC AGG GGC CAC TG-3' (SEQ ID NO:41)

5'-GTC CTC GCA ACT GCG GCG CAG CCG CCG GCC ATG GCC CAG RTG CAG CTG GTG CAR TCT GG-3' (SEQ ID NO:42)

5'-GTC CTC GCA ACT GCG GCG CAG CCG CCG GCC ATG GCC SAG GTC CAG CTG GTR CAG TCT GG-3' (SEQ ID NO:43)

5'-GTC CTC GCA ACT GCG GCG CAG CCG CCG GCC ATG GCC CAG RTC ACC TTG AAG GAG TCT GG-3' (SEQ ID NO:44)

5'-GTC CTC GCA ACT GCG GCG CAG CCG CCG GCC ATG GCC SAG GTG CAG CTG GTG GAG TCT GG-3' (SEQ ID NO:45)

5'-GTC CTC GCA ACT GCG GCG CAG CCG CCG GCC ATG GCC CAG GTG CAG CTG GTG GAG WCY GC-3' (SEQ ID NO:46)

5'-GTC CTC GCA ACT GCG GCG CAG CCG CCG GCC ATG GCC CAG GTG CAG CTA CAG CAG TGG GG-3' (SEQ ID NO:47)

5'-GTC CTC GCA ACT GCG GCG CAG CCG CCG GCC ATG GCC CAG STG CAG CTG CAG GAG TCS GG-3' (SEQ ID NO:48)

5'-GTC CTC GCA ACT GCG GCG CAG CCG CCG GCC ATG GCC GAR GTG CAG CTG GTG CAG TCT GG-3' (SEQ ID NO:49)

5'-GTC CTC GCA ACT GCG GCG CAG CCG CCG GCC ATG GCC CAG GTA CAG CAG CTG CAG TCA GG-3' (SEQ ID NO:50)

FIG. 2iv

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Oligonucleotide primers used for construction of the library

5'-TGA GGA GAC GGT GAC CAG GGT GCC-3' (SEQ ID NO:51)

5'-TGA AGA GAC GGT GAC CAT TGT CCC-3' (SEQ ID NO:52)

5'-TGA GGA GAC GGT GAC CAG GGT TCC-3' (SEQ ID NO:53)

5'-TGA GGA GAC GGT GAC CGT CGT CCC-3' (SEQ ID NO:54)

5'-ACC GCC TCC ACC AGT GCA CTT GAC ATC CAG WTG ACC CAG TCT CC-3' (SEQ ID NO:55)

5'-ACC GCC TCC ACC AGT GCA CTT GAT GTT GTG ATG ACT CAG TCT CC-3' (SEQ ID NO:56)

5'-ACC GCC TCC ACC AGT GCA CTT GAA ATT GTG WTG ACR CAG TCT CC-3' (SEQ ID NO:57)

5'-ACC GCC TCC ACC AGT GCA CTT GAT ATT GTG ATG ACC CAC ACT CC-3' (SEQ ID NO:58)

5'-ACC GCC TCC ACC AGT GCA CTT GAA ACG ACA CTC ACG CAG TCT CC-3' (SEQ ID NO:59)

5'-ACC GCC TCC ACC AGT GCA CTT GAA ATT GTG CTG ACT CAG TCT CC-3' (SEQ ID NO:60)

FIG. 2V



Oligonucleotide primers used for construction of the library

5'-ACC GCC TCC ACC AGT GCA CAG TCT GTG CTG ACT CAG CCA CC-3' (SEQ ID NO:61)
5'-ACC GCC TCC ACC AGT GCA CAG TCT GTG YTG ACG CAG CCG CC-3' (SEQ ID NO:62)
5'-ACC GCC TCC ACC AGT GCA CAG TCT GTC GTG ACG CAG CCG CC-3' (SEQ ID NO:63)
5'-ACC GCC TCC ACC AGT GCA CAR TCT GCC CTG ACT CAG CCT-3' (SEQ ID NO:64)
5'-ACC GCC TCC ACC AGT GCA CTT TCC TAT Gwg CTG ACT CAG CCA CC-3' (SEQ ID NO:65)
5'-ACC GCC TCC ACC AGT GCA CTT TCT TCT GAG CTG ACT CAG GAC CC-3' (SEQ ID NO:66)
5'-ACC GCC TCC ACC AGT GCA CAC GTT ATA CTG ACT CAA CCG CC-3' (SEQ ID NO:67)
5'-ACC GCC TCC ACC AGT GCA CAG GCT GTG CTG ACT CAG CCG TC-3' (SEQ ID NO:68)
5'-ACC GCC TCC ACC AGT GCA CTT AAT TTT ATG CTG ACT CAG CCC CA-3' (SEQ ID NO:69)
5'-ACC GCC TCC ACC AGT GCA CAG RCT GTG GTG ACY CAG GAG CC-3' (SEQ ID NO:70)
5'-ACC GCC TCC ACC AGT GCA CAG CCT GTG ACT CAG CCM CC-3' (SEQ ID NO:71)

FIG. 2vi

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